INTERLABORATORY FISH TOXICITY TEST COMPARISON - AMMONIA

G.R. Craig
Quality Protection Section
Water Resources Branch
July 1983.

INTERLABORATORY FISH TOXICITY TEST COMPARISON - AMMONIA

Table of Contents

alsc

SUMMARY

Seven aquatic toxicity laboratories (federal, provincial and one private) participated in an interlaboratory program to better define the limits of the existing federal aquatic toxicity fish test. The primary issue was the confirmation that fish loading rates in the toxicity test could be lowered to a more manageable level. Secondly the program attempted to identify and quantify sources of variation in the test.

The seven laboratories generated 96 hour LC50's for ammonium chloride using rainbow trout. Tests were completed at two fish loading rates of 0.5 l/g/d and 1.0 l/g/d following the procedures outlined by the protocol. It was concluded that fish loading rates could be reduced to 0.5 l/g/d (from 1.0 l/g/d) without affecting the reliability of the test.

The majority of test variability (96%) was accounted for by water quality indicating that chemical profiles of dilution water were critical to data interpretation.

INTERLABORATORY FISH TOXICITY TEST COMPARISON - AMMONIA

INTRODUCTION

Fish toxicity tests in Canada have been formally applied to industrial effluents since 1971 when the Environment Canada Pulp and Paper Regulations and Guidelines were published. Each new federal industrial effluent guideline carried a modification of the first static toxicity test until 1976. After that time guidelines included a static test protocol which was drafted by an Environment Canada Environmental Protection Service (EPS) Toxicity Technical Co-ordinating Committee during the 1976 Aquatic Toxicity Workshop. The test method was designed according to conventional laboratory practice (in the experience of the Committee) and considered practical for regulatory laboratories across Canada as well as industries or their consultants.

This latest static toxicity test method has been routinely used to estimate the lethality of industrial effluents and commercial products to fish since 1976. The standardized protocol incorporated rainbow trout at a specific loading rate (1.0 l/g/d) and utilized the dilution water available to the testing laboratory. Two practical difficulties were experienced in the application of this test. At certain times of the year the minimum size of rainbow trout available had increased to the point where impractically large volumes of test solution were required to meet the specified fish loading rate. Secondly, there was little information on the effect of dilution water quality on test results produced by different laboratories. This was particularly important when reviewing effluent LC50's (the effluent concentration that kills 50% of the exposed fish) to determine if the respective industrial effluent guideline criteria were met.

The problem concerning fish loading rates was first addressed by Craig and Beggs (1978) indicating that a loading rate of 0.5 l/g/d would produce the same LC50 result as the prescribed loading rate of 1.0 l/g/d in the 96 hour static test using rainbow trout. The second problem could only be addressed through a co-ordinated study involving laboratories using different dilution waters to determine the inherent variation of test results within and among laboratories.

Rather than utilizing a complex toxicant mixture such as an industrial waste, ammonium chloride was selected as the reference toxicant to address the above issues. Ammonium chloride was easily available in reagent grade, exposure concentrations could be analyzed by all participating laboratories and it's toxicity to fish was well documented.

METHODS

The laboratories participating in this program included federal EPS laboratories at St John's, Newfoundland; Burlington, Ontario; Edmonton, Alberta; and Vancouver, British Columbia, provincial laboratories of Alberta Environment, and Ontario Ministry of the Environment and one private consulting laboratory in Vancouver, EVS Consultants. These laboratories followed the prescribed static toxicity testing format (Environment Canada 1977) to estimate the 96 hour LC50 and confidence limits of ammonium chloride for rainbow trout.

Tests were completed using ammonium chloride at fish loading rates of 1.0 and 0.5 l/g/d.

Ammonia was measured by each laboratory either by the colourametric method (Standard Methods 1971) or by specific ion probe (Orion - two cases only). Un-ionized ammonia was calculated according to Emmerson et al (1975). Analytical concentration measurements were used to calculate LC50's.

All laboratories provided a dilution water profile based on measurements of pH, hardness (mg/l $CaCO_3$), alkalinity (mg/l $CaCO_3$), conductivity, Ca, Mg, Na, K, Cl, according to Standard Methods (1971).

All data was transformed logarithmically for ease of statistical analysis, with the exception of pH which was expressed as the log of the hydrogen ion concentration. The influence of the recorded test parameters on LC50's was then determined by regression analysis.

RESULTS and DISCUSSION

Un-ionized ammonia LC50's ranged within a full order of magnitude among labs (Table 1) but confidence limits for the two loading rates (1.0 and 0.5 l/g/d) within labs overlapped in most cases (figure 1). Laboratory 6, had one stray LC50 value (.51 mg/l, loading rate 0.5 mg/l) which may have been attributed to a difference in the pH of the test conditions. Laboratory 3, had two values whose confidence intervals did not overlap (LC50 1.07 mg/l, loading rate 1.02 l/g/d, and LC50 1.44 mg/l, loading rate .54 l/g/d). These two tests were completed on the same day, with identical water quality conditions, using similar sized test fish.

Analysis of variance between LC50's generated with both loading rates indicated similar responses for both 0.5 and 1.0 l/g/d ($p < 0.05$) among all laboratories. In instances where responses were not the same, differences in results were attributed to biological variation.

FIG 1: AMMONIA TOXICITY TEST RESULTS

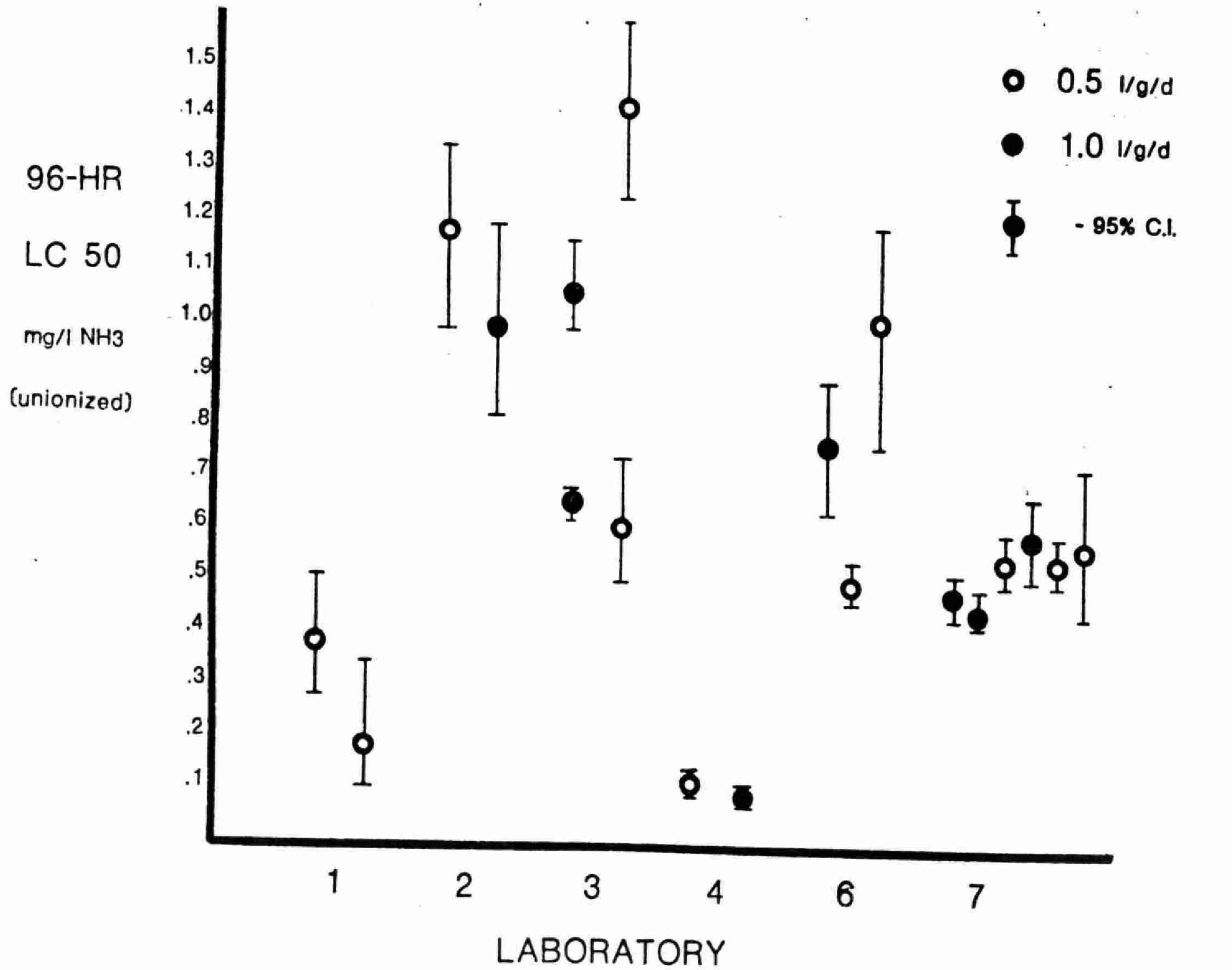

Table 1. Ninety-six hour LC50's of un-ionized ammonia for rainbow trout exposed in different dilution waters (all water quality parameters are measured in mg/l).

Test	LC50	95% C.I.	pH	Alk	Hard	K	Ca	Mg	Na	Loading l/g/d	Fish Size
1	.39	.52-.28	7.1	4	15	.7	7	.6	66	.34	2.9
2	.19	.35-.11	7.1	4	15	.7	7	.6	66	.34	2.9
3	1.18	1.36-1.01	8.0	80	140	1.6	35	6.2	4.2	.42	1.2
4	1.0	1.21-.83	8.0	80	140	1.6	35	6.2	4.2	.81	1.2
5	.67	.69-.64	7.8	93	137	1.6	40	9	12	1.0	.5
6	.62	.75-.51	7.8	93	137	1.6	40	9	12	.5	.6
7	1.07	1.17-.98	8.0	91	143	1.6	44	8	15	1.02	1.0
8	1.44	1.70-1.26	8.0	91	143	1.6	44	8	15	.54	.9
9	.13	.16-.11	6.6	5.1	5.6	.2	1.8	.3	.7	.44	1.7
10	.10	.11-.09	6.6	5.1	5.6	.2	1.8	.3	.7	.84	1.7

Table 1. con't

Test	LC50	95% C.I.	pH	Alk	Hard	K	Ca	Mg	Na	Loading 1/g/d	Fish Size
11	.30	.44-.21	6.3	3.8	8.0	.2	1.8	.3	.7	.91	.6
12	.51	.55-.47	7.2	40	82	.8	20	36	4.9	.2	4.5
13	.76	.90-.64	7.6	40	82	.8	20	36	4.9	2.1	.5
14	1.02	1.2-.87	7.6	40	82	.8	20	36	4.9	.35	2.9
15	.49	.53-.44	7.6	413	105	.9	28	10	6	1.47	.34
16	.46	.5-.43	7.6	413	105	.9	28	10	6	1.35	.37
17	.56	.61-.51	8.0	871	105	.7	20	10	5	.57	.87
18	.59	.68-.51	8.0	871	105	.7	20	11	5	.72	.69
19	.55	.60-.51	8.4	1663	90	.7	17	11	5	.49	1.02
20	.57	.74-.44	8.4	1663	90	.7	17	11	5	.44	1.03

Table 2. Change in the RSQ value when an additional variable was included in the regression analysis.

Additional Variable	*RSQ value*	*Δ RSQ*
K	.7210	.7210
H	.8486	.1280
Alk	.8751	.0265
pH	.8972	.0221
Ca	.9095	.0123
Mg	.9577	.0482
Loading	.9645	.0068
Na	.9672	.0027
Size	.9682	.0010

Test variables together (ie.water quality, fish weight and loading) accounted for 96.8% of the total variation in LC50 results according to regression analysis (Table 2). Water quality parameters alone were responsible for 96% of the observed variation, with 78.4% being contributed by ions. Potassium and magnesium accounted for 72.1% and 4.8% of the variation respectively, while calcium (1.2%) and sodium (.3%) had only a minor influence on LC50 results. Hardness was responsible for 12.8% of the variation, while alkalinity and hydrogen ion concentration accounted for 2.7% and 2.2% of the observed variation. Fish loading and size contributed only .7% and .1% to the total variation and were therefore considered to be negligible. The remaining 3.2% variation is unexplained possibly due to unaccounted biological and analytical factors.

The above illustrates that reported water quality data should not be limited to pH, alkalinity and hardness but should also include the major ionic constituents that contribute to those three characteristics.

The significance of these results for regulatory monitoring is that fish toxicity test variability can be explained within and among laboratories. Water quality is not the only variable as analytical and biological variability is undoubtedly included in the variation expressed here.

These results also raise the question as to the purpose of regulatory tests. Clearly as a first level of protection a fish test performed in any dilution water is sufficient to determine if an effluent is lethal. However it is also evident that if the intention of the test is to protect the receiving environment then the appropriate dilution water must be used for the test. Regulatory or assessment programs must also be tempered with practicality so that a compromise situation of referencing laboratory tests to on-site or receiving water tests may be more manageable for long term monitoring.

Research laboratories should also ensure that toxicity test results be accompanied by a complete dilution water profile for future reference. Biological tests integrate environmental conditions and therefore reflect a lower degree of precision than is generally anticipated from analytical tests therefore differences in biological results among laboratories using different test conditions are to be expected. If water chemistry profiles are provided with toxicological results it is more likely that differences in the biological response among laboratories can be explained.

CONCLUSIONS

1. *The minimum fish loading rate can be reduced to 0.5 l/g/d in the EPS acute static toxicity test without affecting the reliability of the result.*

2. *Toxicity laboratories should routinely monitor dilution water to enable reliable chemical characterization of water quality.*

3. *Complete dilution water profiles should accompany toxicity test results (eg. pH, alkalinity, hardness, Ca, Na, K, Mg, Cl).*

4. *The best estimates of the environmental impact of toxicants in industrial wastes are obtained using receiving water for effluent dilution in toxicity tests.*

5. *The most practical approach in testing chemicals for their aquatic toxicity is to use a variety of water qualities that bracket anticipated receiving water qualities in order to more closely predict toxicity in the receiving environment.*

BIBLIOGRAPHY

Craig G.R. and G.L. Beggs (1979). Evaluation of fish loading rates in regulatory static bioassays. Proc. Fifth Annual Aquatic Toxicity Workshop, Hamilton, Ontario. Fish. Mar. Serv. Tech. Rep. 862 pp. 145-160.

Emmerson K., R.C. Russo, R.E. Lund and R.V. Thurston, (1975). Aqueous ammonia equilibrium calculations: Effects of pH and temperature. J. Fish. Res. Bd. Can. 32: 2379-2383.

Environment Canada, 1980. Standard procedures for testing the acute lethality of liquid effluents. Water Pollution Control Directorate. EPS 1-WP-80-1

Standard Methods for the examination of water and waste water. (1971). Published by the American Public Health Association, American Water Works Association, and the Water Pollution Control Federation. pp.1193.

ACKNOWLEDGEMENTS

The following individuals participated in the interlaboratory testing program and/or review of the manuscript.

Roy Parker	*EPS St. John's, Nfld*
Rick McCubbin	*EPS St. John's, Nfld*
Christine Blais	*EPS Halifax, Nova Scotia*
Keith Holtze	*Ont. Ministry of Environment*
Serge Metacosh	*EPS, Burlington, Ont.*
Bill Lake	*Alberta Environment*
Jackie LeFebvre	*Alberta Environment*
Art Beckett	*EPS Edmonton, Alta*
Ron Watts	*EPS Vancouver, B.C.*
Gary Vigers	*EVS Consultants, B.C.*

The report text and illustrations were completed by Margaret Pike.